Structural Studies of HuD Complexed with AU-Rich Element mRNA

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Introduction: AU-rich elements (AREs) in the 3' untranslated regions of many short-lived mRNAs regulate their stability and play a critical role in the regulation of gene expression during cell growth and differentiation. These AREs have been divided into at least two classes that contain repeats of the sequence AUUUA. The decay of mRNAs containing these two classes of AREs appears to display different kinetics. Hu proteins bind to both class I and class II AREs and stabilize the ARE-containing mRNA. Hu proteins contain three RNA recognition motif (RRM) domains, a specific RNA binding module in a variety of RNA-binding proteins. The first two RRM domains are in tandem and bind to AREs, and the third RRM domain occurs after a spacer region and may bind to long poly-(A) tails. Four Hu proteins have been identified. HuR is expressed in all tissues while Hel-N1, HuC, and HuD are expressed in neurons and are target antigens in an autoimmune neural degeneration associated with small cell lung cancer.

Methods and Materials: In order to understand how Hu proteins specifically recognize their RNA partners and examine how the protein-RNA interactions may stabilize the mRNA, we determined a 1.8 Å crystal structure of an ARE-binding fragment of HuD (HuD1,2) in complex with an 11-nt fragment of the cfos ARE (cfos-11), a class I ARE. The structure of the HuD1,2:cfos-11 complex was determined by molecular replacement using the structure of the two RRM domains of Sex lethal (Sxl) protein as a search model combined with an iodine derivative.

Results: Each RRM domain contains a four-stranded beta sheet and two alpha helices (Figure 1). The two RRM domains form a cleft with the cfos-11 RNA bound between the basic surfaces of the opposing beta sheets. The RNA contains no base pairs and is in an extended conformation with a turn centered at U5. HuD1,2 provides an extensive surface for RNA binding and recognition.

Conclusions: Approximately 50% of the RNA molecular surface area is buried as a result of protein:RNA interaction and may protect ARE-containing mRNAs from ARE-specific nucleases and increase the lifetime of the mRNAs. The structure reveals a consensus RNA recognition sequence that suggests a preference for pyrimidine-rich sequences and a requirement for a central uracil residue in the clustered AUUUA repeats found in class II AREs. Comparison to structures of other RRM domain:nucleic acid complexes reveals two base recognition pockets in all structures that interact with bases using residues in conserved ribonucleoprotein motifs and at the C-terminal ends of RRM domains (Figure 2). Different conformations of nucleic acid can be bound by RRM domains by using different combinations of base recognition pockets and multiple RRM domains.

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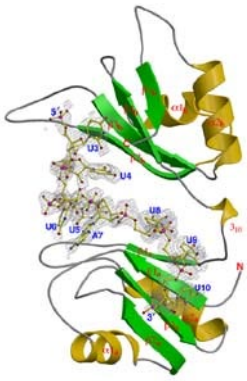

Figure 1. Ribbon diagram of the HuD1,2:cfos-11 complex. β strands are colored green, helices are colored gold, and the RNA is shown as a ball-and-stick model colored by atom type (nitrogen = blue, carbon = yellow, oxygen = red, phosphorus = magenta). The N- (S37) and C- (A203) termini of the protein and bases and 5' and 3' ends of the RNA are labeled. A simulated annealing omit map for the cfos-11 RNA contoured at 1σ is shown superimposed on the ball-and-stick model.

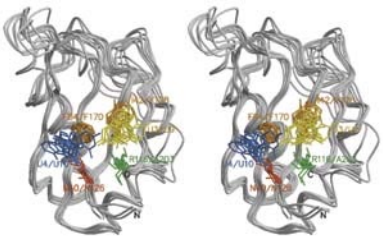

Figure 2. Nucleic Acid Recognition by RRM Domains. Stereo diagram of superposition of RRM domains from HuD, Sxl, PABP, UP1, U1A, and U2B". Bases in all structures at positions equivalent to U3/U9 and U4/U10 in the HuD1,2:cfos-11 structure are shown in yellow and blue, respectively. Amino-acid side chains at positions equivalent to N40/N126, I42/Y128, and F84/F170 in HuD1,2 are shown and backbone atoms at positions equivalent to R116/A203 are shown. Residues that interact with the bases through a stacking interaction are colored orange, residues that interact through the side chain are colored red, and residues that interact through the main chain are colored green.